MEDICOVER GENETICS

PATIENT INFORMATION

NAME
SVETOSLAVA LAZAROVA

PATIENT GENETIC SEX
FEMALE

PATIENT ETHNICITY

DATE OF BIRTH
07/07/1978

REFERRAL INFORMATION

CLINIC NAME
SYNEVO BULGARIA

CLINIC ID

CLINIC EMAIL

REFERRING HEALTHCARE PROVIDER
DR. STOYAN BICHEV

TEST INDICATIONS

DESCRIPTION

ICD-10 CODE

UROTHELIAL CARCINOMA

SAMPLE INFORMATION

ORDER NUMBER	LAB NUMBER	DATE OF COLLECTION	DATE RECEIVED
N354915	**401948**	**02/03/2026**	**03/03/2026**

TEST PERFORMED ForeSENTIA Pan-Cancer panel PLUS

For a full list of evaluated diseases and genes tested please refer to: **bit.ly/FS-Plus-Genes-260224**

TEST RESULTS

Clinically significant variant(s) detected

BIOMARKER FINDINGS

BIOMARKER	FDA/EMA APPROVED THERAPIES (IN PATIENT'S INDICATIONS)*	CLINICAL TRIALS
MSI-H: Not detected	None	10
TMB: 1.18 mut/Mb	None	8

GENOMIC FINDINGS

GENE	VARIANT DETECTED	ALLELE FRACTION	FDA/EMA APPROVED THERAPIES (IN PATIENT'S INDICATIONS)*	FDA/EMA APPROVED THERAPIES (IN OTHER INDICATIONS)**	CLINICAL TRIALS
TP53	NM_000546.6: c.216del (p.Val73TrpfsTer50)	20.6%	None	None	1
TP53	NM_000546.6: c.437G>A (p.Trp146Ter)	8.3%	None	None	1
PIK3CA	NM_006218.4: c.1633G>A (p.Glu545Lys)	19.0%	None	Alpelisib Capivasertib Inavolisib	0
KDM6A	NM_001291415.2:	9.6%	None	None	2

c.1261G>T
(p.Glu421Ter)

Approved in indication *Lack of response* *Included in NCCN guideline*

⚠ Genetic findings in this report are associated with conflicting evidence regarding the potential effect of the treatment.

'List of FDA[1] and/or EMA[2] approved drugs in the patient's cancer type
''List of FDA and/or EMA approved drugs in other tumor types. Therapies that are included in the NCCN guidelines[3] for the patient's cancer type are clearly indicated above.
Note: Clinical trials listed in this report are retrieved from clinicaltrials.gov[4] and only include active, enrolling by invitation and recruiting trials for the indicated cancer type and gene. The list of therapies and clinical trials included in this report may not be complete and/or exhaustive. Therapies contained in this report are FDA/EMA approved, however information on drug approvals for different indications is updated regularly, based on new evidence, and may not reflect the current status at any time. This report should not be used as the sole basis when making treatment decisions, instead it should be regarded as a supplementary source of information for guiding therapy decisions. All treatment decisions remain the full and final responsibility of the treating clinician.

INTERPRETATION

BIOMARKER FINDINGS

MSI-H
Not detected
There is insufficient evidence of genomic instability at the microsatellite regions tested in the patient's sample.

TMB
Low
Tumor mutational burden (TMB) is defined as the number of somatic mutations per megabase of the interrogated coding sequence[5]. The number of mutations accumulated is associated with the neoantigen load on tumor cell surface, which makes the tumor more sensitive to immune checkpoint inhibitors (ICIs) such as anti-PD-1 and anti-PD-L1 therapies[6]. The higher the neoantigen load or TMB score the more chances there are to achieve significant clinical benefit from treatment with ICIs[7]. TMB score lower than 10 mutations per megabase is considered as TMB-low. TMB low score is associated with lower rates of clinical benefit from treatment with immune checkpoint inhibitors compared with patients with tumors harboring higher TMB levels, based on clinical studies in a wide spectrum of solid tumor malignancies[1,8].

For more information on clinical trials related to the biomarker findings (MSI and TMB) for patients with urothelial carcinoma, please visit clinicaltrials.gov[4].

GENOMIC FINDINGS

Variant Detected
TP53 NM_000546.6:c.216del (p.Val73TrpfsTer50) & c.437G>A (p.Trp146Ter)

Variant Details
c.216del (p.Val73TrpfsTer50)
The TP53 variant c.216del is classified as a Tier 2 variant of potential clinical significance in urothelial carcinoma[9]. This is a frameshift variant in exon 4 of the TP53 gene (NM_000546.6) that results in a valine to tryptophan substitution at position 73 of the protein sequence and the introduction of a premature stop codon at position 50 of the new open reading frame of the protein. This variant occurs in a region of the protein that is critical for the interaction with other proteins such as CCAR2, HRMT1L2 and WWOX[10]. This variant has been previously reported in several cancer types including bladder and submitted in the COSMIC (COSV52692730) and IARC TP53 somatic cancer databases[11,12].

c.437G>A (p.Trp146Ter)
The TP53 variant c.437G>A is classified as a Tier 2 variant of potential clinical significance in urothelial carcinoma[9]. This is a nonsense variant in exon 5 of the TP53 gene (NM_000546.6) which results in the introduction of a premature stop codon at position 146 of the protein sequence and is predicted to result in an absent or disrupted protein product. This variant lies within the DNA-binding region of the protein, a region that is critical for the interaction with other proteins such as HIPK1, CCAR2, AXIN1, ZNF385A and FBXO42[10]. Although it has not been biochemically characterized, due to the effects of truncation mutations downstream of W146, it is predicted to lead to a loss of Tp53 protein function[13]. This variant has been previously reported in several cancer types including bladder and submitted in the COSMIC (COSV52661900) and IARC TP53 somatic cancer databases[11,12].

Gene information and significance

The TP53 gene encodes for the TP53 protein, which acts as a tumor suppressor protein regulating DNA repair and cell division. When the DNA is damaged, TP53 plays a critical role in determining whether the DNA will be repaired, or if the damaged cell will undergo apoptosis. If the DNA damage can be repaired, TP53 activates DNA repair proteins to correct the DNA damage. If the DNA damage cannot be repaired, then TP53 prevents the cell from undergoing cell division and induces programmed cell death (apoptosis), eventually preventing the development of tumors[14]. TP53 mutations occur across cancer types. The loss of a tumor suppressor is most often through large deleterious events, such as frameshift mutations, or premature stop codons. However, many of the observed mutations in cancer are found to be single nucleotide missense variants. These variants are broadly distributed throughout the gene, but with the majority localizing in the DNA binding domain. These mutations either directly disrupt the DNA-binding domain of TP53 or cause conformational changes of the TP53 protein, thus leading to severely impaired TP53 function[15,16]. Mutations in the TP53 gene occur in 36% of urinary tract carcinoma cases (2058 mutated/5770 total cases) submitted in the COSMIC database[11]. TP53 mutations may be potential prognostic and predictive biomarkers in some tumor types as well as targets for pharmacological intervention in some clinical settings[16].

Approved therapies and available clinical trials

Currently, there are no approved therapies for urothelial carcinoma patients with TP53 mutations. One clinical trial is currently recruiting urothelial carcinoma patients with TP53 status as an indicator. For more information on clinical trials please visit clinicaltrials.gov[4].

Variant Detected

PIK3CA NM_006218.4:c.1633G>A (p.Glu545Lys)

Variant Details

The PIK3CA variant c.1633G>A is classified as a Tier 2 variant of potential clinical significance in urothelial carcinoma[9]. This is a missense variant in exon 10 of the PIK3CA gene (NM_006218.4) which results in an amino acid change from glutamic acid to lysine at position 545 of the protein sequence (E545K). This hotspot mutation lies within the PIK helical domain of the Pik3ca protein and results in increased phosphorylation of Akt and Mek1/2, growth factor-independent cell survival, and transformation in cell culture[10,13]. This variant has been previously reported in urinary tract, colorectal and breast cancers and submitted in the COSMIC (COSV55873239) somatic cancer database[11].

Gene information and significance

The PIK3CA gene encodes for the p110 alpha subunit of the phospatidylinositol3-kinase (PIK3). The 110a protein is the catalytic subunit of the enzyme while the other subunits regulate the enzyme's activity. PI3K activates signaling pathways involved in cell growth, survival, proliferation, motility and morphology[10]. PIK3CA mutations activate the PI3K-PTEN-AKT pathway which is downstream from both the EGFR and the RAS-RAF-MAPK pathways. The somatic mutations found thus far in PIK3CA are oncogenic, and the majority of them are clustered within exon 10 and 21 (helical and kinase domains), with 80% of the identified mutations found within three hotspots: Glu542Lys, Glu545Lys, and His1047Arg[16,14]. Mutations in the PIK3CA gene occur in 20% of urinary tract carcinoma cases (690 mutated/3413 total cases) submitted in the COSMIC database[11].

Approved therapies and available clinical trials

Currently, there are no approved therapies or any available clinical trials for urothelial carcinoma patients with PIK3CA mutations. However, alpelisib, capivasertib, and inavolisib + palbociclib, each in combination with the selective estrogen receptor degrader (SERD) fulvestrant, are FDA-approved for the treatment of HR+/HER2- metastatic breast cancer patients with select activating PIK3CA mutations[1,2,17]. The clinical utility of these combinations in patients with PIK3CA-altered urothelial carcinoma is unknown[17]. For more information on clinical trials please visit clinicaltrials.gov[4].

Variant Detected

KDM6A NM_001291415.2:c.1261G>T (p.Glu421Ter)

Variant Details

The KDM6A variant c.1261G>T is classified as a Tier 2 variant of potential clinical significance in urothelial carcinoma[9]. This is a nonsense variant in exon 13 of the KDM6A gene (NM_001291415.2) which results in the introduction of a premature stop codon at position 421 of the protein sequence and is predicted to result in an absent or disrupted protein product. This variant has not been reported in a somatic cancer database such as the COSMIC[11]. However, this is a truncating mutation in a tumor suppressor gene, and therefore is likely oncogenic[17].

Gene information and significance

The KDM6A (lysine-specific demethylase 6A) gene encodes a chromatin-modifying enzyme that mediates transcriptional co-activation by functioning as a di- and tri-methylated histone H3 lysine 27 (H3K27) demethylase[17]. KDM6A is part of the larger ASC-2 complex (ASCOM) that also contains KMT2D and KMT2C[17]. Association of KDM6A with KMT2D and KMT2C couples

H3K27 demethylation to H3K4 methylation[18]. Germline deletions and point mutations in KDM6A cause Kabuki syndrome[19-22]. Inactivating KDM6A mutations have been found in a number of human malignancies including multiple myeloma, esophageal squamous cell carcinoma, clear cell renal cell carcinoma, medulloblastoma, adenoid cystic carcinoma, urothelial bladder cancer, aristolochic acid-associated upper tract urothelial carcinoma, T-cell acute lymphoblastic leukemia, and pancreatic cancer[23-34]. Mutation in the KDM6A gene have been reported in 29% of urinary tract carcinoma cases (551 mutated/1871 total cases) submitted in the COSMIC database[11]. Laboratory data suggest that cancer cells with loss-of-function KDM6A alterations may be sensitive to EZH2-targeted inhibitors such as tazemetostat[17,35].

Approved therapies and available clinical trials

Currently, there are no approved therapies for urothelial carcinoma patients with KDM6A mutations. A few clinical trials are currently active or recruiting urothelial carcinoma patients with KDM6A status as an indicator. For more information on clinical trials please visit clinicaltrials.gov[4].

Results should be interpreted in conjunction with other laboratory and clinical findings.

VARIANTS OF UNKNOWN SIGNIFICANCE

GENETIC ALTERATION	ALLELE FRACTION
KMT2D NM_003482.4:c.13745_13761delinsA (p.Gly4582AspfsTer5)	25.3%
KMT2D NM_003482.4:c.9805_9824del (p.Gln3269AlafsTer25)	10.8%
PIK3CA NM_006218.4:c.1108_1119delinsCTGG (p.Asn370LeufsTer13)	11.1%
DICER1 NM_177438.3:c.347A>C (p.His116Pro)	18.9%
MPL NM_005373.3:c.199T>C (p.Tyr67His)	25.3%

Note: One or more variants of unknown significance (VUS) have been detected in this patient's sample. These variants are known as VUS due to their limited characterization and clinical evidence in the scientific literature at the time of writing of this report, making their significance unclear. However, we do include them here for reference in case they become clinically important in the future.

METHODOLOGY / LIMITATIONS

ForeSENTIA is a Laboratory Developed Test (LTD) from Medicover Genetics Ltd for tumor molecular testing. Genomic deoxyribonucleic acid (gDNA) is extracted using a standardized methodology and subjected to enzymatic fragmentation and DNA library preparation. DNA enrichment for the genomic regions of interest is carried out using a solution-based hybridization method followed by next generation sequencing (NGS). Sequence data is aligned to a reference genome and variants are identified using proprietary bioinformatics pipelines. ForeSENTIA can be used for the identification of selected single nucleotide variants (SNVs), small insertions and deletions (Indels, ≤50bp), rearrangements, copy number alterations (CNAs), Microsatellite Instability (MSI) and Tumor Mutational Burden (TMB). Tumor-related actionable and clinically relevant alterations are reported. Analysis and Interpretation is performed using but not limited to Varsome Clinical platform according to published knowledge at the time of testing and based on recommendations of the Association for Molecular Pathology (AMP)[9].

This test detects SNVs, Indels, CNAs, and other structural rearrangements in the targeted regions in genes relevant to cancer development covered by the assay. The MSI and TMB biomarkers are also tested aiming to identify immunotherapy eligibility. Variants that fall outside of the targeted bases are not intended to be detected by this assay. Sequence alterations such as SNVs and Indels are evaluated when the variant allele frequency is at least 5% and 10% respectively with 100% sensitivity (95% CI:96-100%) and 100% specificity (95% CI: 92-100%). In certain cases - depending on the sample quality metrics - variants with a VAF <5% may be reported. Rearrangements are reported when the frequency is at least 20% with estimated sensitivity and specificity of 100% (95% CI:66-100%) and 100% (95% CI:72-100%) respectively. CNAs are evaluated when the copy number amplification is either ≥4-fold with tumor DNA purity being >30% or ≥6-fold with tumor DNA purity at 20%. Biallelic deletions in the genes tested for CNAs are evaluated when the tumor fraction is higher than 50%. The sensitivity and specificity of the assay for CNAs are 100% (95% CI: 79-100%) and 100% (95% CI: 96-99.8%) respectively. The MSI status is reported when VAF≥5% for indels at selected microsatellite regions with an estimated sensitivity and specificity of 100% (95% CI:85-100%) and 100% (95% CI:80-100%) respectively. The TMB scoring system was developed and validated using variants with a VAF≥5%, which had previously been confirmed by an orthogonal method. The concordance correlation coefficient for the TMB score was 0.84 (p<0.0001).

A 'clinically significant variant detected' result indicates the presence of a clinically relevant alteration. Classification and interpretation of variants is according to published knowledge at the time of reporting. Variants which are classified as variants of strong clinical significance (Tier I) or variants of potential clinical significance (Tier II) are reported and interpreted. Variants of unknown significance (Tier III) are also reported. Variants which have been detected and are classified as benign or likely benign (Tier IV) are not reported. Genetic counselling for clinical interpretation and significance of the results is recommended. A 'no clinically significant variant detected' result reduces the chance of presence but does not guarantee the absence of a somatic variant in the patient's tumor.

Reduced sensitivity for the detection of the targeted genetic alterations may be due to low quality of the sample because of the procedure of tissue formalin-fixation or other factors that include, but are not limited to, low DNA yield, insufficient tumor DNA content and high intratumor heterogeneity in the specimen provided. Certain genetic alterations in targeted regions containing repeats, sequences of high homology such as segmental duplications and pseudogenes, as well as regions of high/low GC-content, low mappability or for other technical reasons, may have reduced coverage. The test does not determine whether a variant is somatic or germline. Patients with an alteration identified in genes that are also associated with cancer predisposition may benefit from additional germline testing.

Test performance is valid only for the presence or absence of cancer-associated variants in the genomic regions targeted by this test. Therefore, a 'no clinically significant variant detected' result indicates the absence of a cancer variant out of all the variants in the genomic regions covered by the test above the specified threshold for detection and does not eliminate the possibility of a variant in a genomic position not tested by this assay. The results are interpreted based on information provided on the sample information form. Misinterpretation of results may occur if insufficient or inaccurate information is provided. A 'clinically significant variant detected' result does not guarantee association with a certain treatment or drug. Drugs or treatments mentioned in this report may not necessarily be suitable for the patient. Decisions on medical management must be based on the clinician's judgement, taking into consideration all available information such as the patient's medical history, family history and other medical tests and examinations performed.

The ForeSENTIA test development and performance evaluation were carried out by Medicover Genetics Ltd, which is accredited by the College of American Pathologists (CAP) and certified with ISO 15189:2022 as qualified to perform high-complexity testing. ForeSENTIA is intended for clinical purposes and should not be regarded as investigational or for research. The test has not been cleared or approved by the U.S. Food and Drug Administration (FDA), which does not require this test to go through premarket FDA review.

ADDITIONAL INFORMATION / DISCLOSURE

Validation studies are carried out by Medicover Genetics Ltd. Although this test is highly accurate, there is still a small possibility for false positive or false negative results. This may be caused by technical and/or biological limitations, including but not limited to poor sample quality, bone marrow transplants or other rare molecular events. Other reasons for false positive or false negative results include, but are not limited to mislabeled samples, inaccurate reporting of clinical/medical information and rare technical errors. Genetic testing is an important part of the diagnostic process. However, genetic tests may not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in current medical knowledge or testing technology. Clinical correlation with other clinical data and tests is recommended. Results should always be considered in the context of other clinical criteria. The analysis is specific only for the test ordered. The referral clinician is responsible for counselling before and after the test including the provision of advice regarding the need for additional genetic testing. Other diagnostic tests may still be necessary.

REFERENCES

1. https://www.fda.gov. FDA.
2. https://www.ema.europa.eu. EMA.
3. National Comprehensive Cancer Network.
4. Health, A. service of the U. S. N. I. of. Clinical trials.gov. *U.S. National Institutes of Health* (2015).
5. Vega, D. M. *et al.* Aligning tumor mutational burden (TMB) quantification across diagnostic platforms: phase II of the Friends of Cancer Research TMB Harmonization Project. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **32**, 1626–1636 (2021).
6. Merino, D. M. *et al.* Establishing guidelines to harmonize tumor mutational burden (TMB): in silico assessment of variation in TMB quantification across diagnostic platforms: phase I of the Friends of Cancer Research TMB Harmonization Project. *J. Immunother. cancer* **8**, (2020).
7. Stenzinger, A. *et al.* Tumor mutational burden standardization initiatives: Recommendations for consistent tumor mutational burden assessment in clinical samples to guide immunotherapy treatment decisions. *Genes. Chromosomes Cancer* **58**, 578–588 (2019).

8. Goodman, A. M. *et al.* Tumor Mutational Burden as an Independent Predictor of Response to Immunotherapy in Diverse Cancers. *Mol. Cancer Ther.* **16**, 2598–2608 (2017).
9. Li, M. M. *et al.* Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *Journal of Molecular Diagnostics* at https://doi.org/10.1016/j.jmoldx.2016.10.002 (2017).
10. Bateman, A. *et al.* UniProt: The universal protein knowledgebase. *Nucleic Acids Res.* (2017) doi:10.1093/nar/gkw1099.
11. Forbes, S. A. *et al.* COSMIC: Somatic cancer genetics at high-resolution. *Nucleic Acids Res.* **45**, D777–D783 (2017).
12. Bouaoun, L. *et al.* TP53 Variations in Human Cancers: New Lessons from the IARC TP53 Database and Genomics Data. *Hum. Mutat.* **37**, 865–876 (2016).
13. Patterson, S., Statz, C., Yin, T. & Mockus, S. The JAX Clinical Knowledgebase: A Valuable Resource for Identifying Evidence Related to Complex Molecular Signatures in Different Types of Cancer. *Cancer Genet.* **214–215**, 33 (2017).
14. MedlinePlus [Internet]. Bethesda (MD): National Library of Medicine (US). Genetics Home Reference. https://medlineplus.gov/.
15. Griffith, M. *et al.* CIViC is a community knowledgebase for expert crowdsourcing the clinical interpretation of variants in cancer. *Nat. Genet.* **49**, 170–174 (2017).
16. Huang, L. *et al.* The cancer precision medicine knowledge base for structured clinical-grade mutations and interpretations. *J. Am. Med. Informatics Assoc.* (2017) doi:10.1093/jamia/ocw148.
17. Chakravarty, D. *et al.* OncoKB: A Precision Oncology Knowledge Base. *JCO Precis. Oncol.* **2017**, 1–16 (2017).
18. Lee, M. G. *et al.* Demethylation of H3K27 Regulates Polycomb Recruitment and H2A Ubiquitination. doi:10.1126/science.1149042.
19. Lederer, D. *et al.* Deletion of KDM6A, a Histone Demethylase Interacting with MLL2, in Three Patients with Kabuki Syndrome. doi:10.1016/j.ajhg.2011.11.021.
20. Priolo, M. *et al.* Absence of deletion and duplication of MLL2 and KDM6A genes in a large cohort of patients with Kabuki syndrome. *Mol. Genet. Metab.* **107**, 627–629 (2012).
21. Banka, S. *et al.* MLL2 mosaic mutations and intragenic deletion–duplications in patients with Kabuki syndrome. *Clin. Genet.* **83**, 467–471 (2013).
22. Miyake, N. *et al.* KDM6A Point Mutations Cause Kabuki Syndrome. *Hum. Mutat.* **34**, 108–110 (2013).
23. van Haaften, G. *et al.* Somatic mutations of the histone H3K27 demethylase, UTX, in human cancer Europe PMC Funders Group. *Nat Genet* **41**, 521–523 (2009).
24. Waddell, N. *et al.* Whole genomes redefine the mutational landscape of pancreatic cancer. *Nature* **518**, 495–501 (2015).
25. Jones, D. T. W. *et al.* ICGC PedBrain: Dissecting the genomic complexity underlying medulloblastoma. *Nature* **488**, 100 (2012).
26. Varela, I. *et al.* Exome sequencing identifies frequent mutation of the SWI/SNF complex gene PBRM1 in renal carcinoma. *Nature* **469**, 539 (2011).
27. Van Der Meulen, J. *et al.* The H3K27me3 demethylase UTX is a gender-specific tumor suppressor in T-cell acute lymphoblastic leukemia. *Blood* **125**, 13 (2014).
28. Poon, S. L. *et al.* Genome-wide mutational signatures of aristolochic acid and its application as a screening tool. *Sci. Transl. Med.* **5**, (2013).
29. Nickerson, M. L. *et al.* Concurrent Alterations in TERT, KDM6A, and the BRCA Pathway in Bladder Cancer. *Clin. Cancer Res.* **20**, 4935–4948 (2014).
30. Kim, P. H. *et al.* Genomic Predictors of Survival in Patients with High-Grade Urothelial Carcinoma of the Bladder. *Eur. Urol.* **67**, 198 (2014).
31. Gui, Y. *et al.* Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat. Genet.* **43**, 875 (2011).
32. Weinstein, J. N. *et al.* Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature* **507**, 315 (2014).
33. Ho, A. S. *et al.* The Mutational Landscape of Adenoid Cystic Carcinoma. *Nat. Genet.* **45**, 791 (2013).
34. Robinson, G. *et al.* Novel mutations target distinct subgroups of medulloblastoma. *Nature* **488**, 43 (2012).
35. Ler, L. D. *et al.* Loss of tumor suppressor KDM6A amplifies PRC2-regulated transcriptional repression in bladder cancer and can be targeted through inhibition of EZH2. *Sci. Transl. Med.* **9**, (2017).

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Date of report (DD/MM/YYYY): **30/03/2026**

For a full list of evaluated diseases and genes tested please refer to: **bit.ly/FS-Plus-Genes-260224**